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Evidence for Two Species of Glutamate Dehydrogenases in *Thiobacillus novellus*

H. B. LÉJOHN AND BARBARA-ELLEN McCREA[1]
Department of Microbiology, University of Manitoba, Winnipeg 19, Manitoba, Canada

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When grown autotrophically in a thiosulfate-mineral salts medium, cells of the facultative chemoautotrophic bacterium, *Thiobacillus novellus*, produced two distinct glutamate dehydrogenases, one specific for nicotinamide adenine dinucleotide phosphate (NADP) and the other specific for nicotinamide adenine dinucleotide (NAD). When glutamate was supplied exogenously as the sole carbon source, the NAD-specific glutamate dehydrogenase was fully induced. Lower levels of the enzyme were found in bacteria grown in L-arginine, L-alanine, glucose, glycerol, lactate, citrate, or succinate. Arginine, histidine, and aspartate, on the other hand, caused a marked repression of the NADP-specific glutamate dehydrogenase activity. The NAD-dependent glutamate dehydrogenase was allosteric. Adenosine-5'-monophosphate and adenosine-5'-diphosphate acted as positive effectors. Both glutamate dehydrogenases were purified about 250-fold and were shown to be distinct protein with different physical properties.

It has been known for some time that the facultative chemoautotroph, *Thiobacillus novellus*, can grow rapidly on glutamate as a heterotrophic organism (10). In addition, *T. novellus* can be cultured as an autotroph in a medium composed entirely of mineral salts, when thiosulfate or some other reduced inorganic sulfur compound supplies energy and CO_2 is fixed via the Calvin-Benson pathway. In addition, glutamate can serve as a nitrogen and carbon source in a nitrogen-free medium (LéJohn, *unpublished data*). It is reasonable to assume that *T. novellus* possesses an active glutamate dehydrogenase [L-glutamate-NAD(P) oxidoreductase EC 1.4.1.3] that can catalyze the reversible breakdown of glutamate to provide the necessary carbon and nitrogen required for growth in glutamate. It seems logical then to expect that this enzyme, which is at an important cross-link of carbohydrate and nitrogen metabolism, is regulated in some way. Either there are two enzymes, each acting independently, favoring synthesis or breakdown or, alternatively, the same enzyme is under some form of regulation.

Glutamate dehydrogenases (GDH) have been widely studied in plants, animals, and fungi. A detailed review of this appeared recently (3). There is, as yet, relatively little information available on the GDH of bacteria. A widespread belief is that bacteria possess only one type of GDH, which is either specific for nicotinamide adenine dinucleotide (NAD) or for nicotinamide adenine dinucleotide phosphate (NADP). This report clearly shows that, in *T. novellus*, there are two distinct GDH, one specific for NAD and the other specific for NADP. The NAD-linked enzyme is inducible and allosteric (6).

[1] Predoctoral Fellow.

MATERIALS AND METHODS

Bacteria and culture conditions. A strain of *T. novellus* (ATCC 8093) was grown and harvested as described elsewhere (7). Bacterial cells were cultured in a glutamate-thiosulfate mineral salts medium to isolate the two GDH. During induction studies, the pertinent carbon source was substituted for glutamate.

Protein determination. Protein content was determined spectrophotometrically (11) and by the phenol method of Lowry et al. (8).

Growth. Growth was determined by measuring the optical density (OD) of the cells at 530 mμ by use of a Klett-Summerson photoelectric colorimeter (the cells were first filtered through loosely packed cotton plugs to remove colloidal sulfur that is deposited during growth).

Preparation of cell-free extracts. The pellet of harvested bacteria was resuspended as a 25 to 30% (wet weight/volume) suspension of cells in 0.05 M tris-(hydroxymethyl)aminomethane chloride (Tris chloride), *p*H 8, containing 10^{-3} M reduced glutathione (GSH) and 10^{-4} M adenosine-5'-monophosphate (AMP) (designated TGA-buffer). The cells were disrupted in a 10-kc Raytheon sonic disintegrator (Raytheon Co., South Norwalk, Conn.) for 30 min at 5 C. Cell debris was removed by centrifugation at 48,000 × *g* for 15 min at 2 C. Occasionally, particulate reduced

NAD ($NADH_2$) oxidase was removed by centrifuging in a Spinco model L ultracentrifuge (Beckman Instruments Inc., Fullerton, Calif.) at 105,000 × *g* for 2 hr, and the red supernatant solution was used for estimating enzyme content in crude extracts.

Enzyme assays. GDH were assayed by measuring either the oxidation or the reduction of the appropriate NAD at 340 mμ in a Gilford model 2000 multiple recording spectrophotometer. One unit of enzyme activity was defined as that amount of enzyme which caused an OD increase or decrease of 0.001 in 1 min at 340 mμ. Although the GDH activities were assayed in both directions of equilibrium, the results reported for the isolation of the enzymes were expressed in terms of the oxidation of $NADH_2$ or reduced NADP ($NADPH_2$). In the induction studies, the assay medium consisted of 200 mM glutamate, 2 mM NAD(P), extract taken at Stage 2 (*see* Table 1), and 173 mM Tris chloride buffer at *p*H 9.5 (NAD-specific GDH) and at *p*H 9.0 (NADP-specific GDH), in a total volume of 3 ml.

In routine assays of NAD-specific GDH, the reaction system in the oxidative deamination procedure contained 50 mM glutamate, 2 mM NAD, 167 mM Tris chloride (*p*H 9.5), and enzyme, in a total volume of 3 ml. In the reductive amination assay system, the reaction mixture contained 16.66 mM α-ketoglutarate, 0.2 mM $NADH_2$, 40 mM ammonium sulfate, 165 mM Tris chloride (*p*H 8), and enzyme, in a total volume of 3 ml.

For NADP-specific GDH, the reaction systems in both directions of assay were the same as for the NAD-specific GDH, with the exception that NADP and $NADPH_2$ were substituted for NAD and $NADH_2$, respectively. The *p*H levels of the reactions were 9.0 and 7.5. All the reaction rates were linear and proportional to the enzyme concentration in any of these assay conditions.

Glutamate-aspartate transaminase activity was measured by use of malic dehydrogenase as the indicator enzyme according to the procedure of Bergmeyer and Bernt (2).

Preparation of diethylaminoethyl (DEAE) cellulose. DEAE cellulose was cleaned and prepared for use as described by Peterson and Sober (9).

Reagents and chemicals. Medium mesh (0.90 meq/g) DEAE cellulose was purchased from Sigma Chemical Co., St. Louis Mo. NAD, NADP, enzymatically reduced $NADH_2$ and $NADPH_2$, deoxyribonuclease (five times crystallized, protease- and salt-free), GSH, malic dehydrogenase, AMP, adenosine-5′-triphosphate (ATP), adenosine-5′-diphosphate (ADP), and α-ketoglutarate (Na and K salts) were also purchased from Sigma Chemical Co. All other reagents and chemicals used in this study were of the highest grade available commercially.

RESULTS

There were five stages in the purification of GDH.

Stage 1. The red supernatant fluid (*see* Materials and Methods) was treated with deoxyribonuclease (50 μg/ml) and ribonuclease (50 μg/ml) and then incubated at 37 C for 30 min. The suspension was centrifuged at 48,000 × *g* for 15 min, and the top four-fifths of the supernatant fluid was carefully removed. This solution was brought to 55 C within 2 min, held at that temperature for 3 min, and then chilled in an ice-salt bath. After the viscous paste was made to 3 volumes with ice-cold 0.05 M TGA buffer (*p*H 7.5) and thoroughly mixed, the denatured proteins were removed by centrifugation. The supernatant fluid was saved and the residue was discarded. From this point on, all operations were carried out at 5 C.

Stage 2. Column Chromatography. A column of DEAE cellulose (30 × 2.5 cm) was packed under gravity and equilibrated with the starting buffer (0.05 M TGA, *p*H 7.5). A 100-ml amount of the extract was adsorbed to the column in 5-ml portions. The column was washed with 500 ml of the starting buffer to remove unadsorbed proteins. A clear yellow band of flavoproteins remained associated with the enzymes (brown tint) at the top of the column. This served as a convenient marker for routine assays of the fractions containing the GDH enzymes. The enzymes were eluted by use of a linear gradient of 0.05 to 0.5 M TGA buffer (*p*H 7.5). Fractions (6 ml) were collected automatically. The enzyme fractions showing the highest activities toward NADP (peak I in Fig.

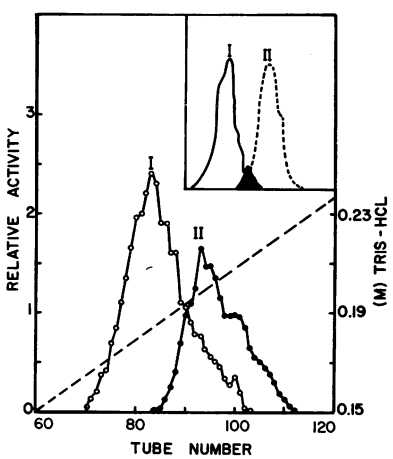

FIG. 1. *DEAE cellulose chromatogram of two glutamate dehydrogenases of Thiobacillus novellus (ATCC 8093). Enzyme activities were determined by measuring the oxidation of both $NADPH_2$ and $NADH_2$ in the defined assay mixtures (see Materials and Methods), by use of 0.2 ml of the enzyme fractions. The linear salt gradient (dashed line) was determined colorimetrically in a separate experiment. The profiles in the inset show the elution patterns of the NADP-specific (peak I) and NAD-specific (peak II) glutamate dehydrogenase, after rechromatography on DEAE cellulose. The shaded area indicates those fractions that had both NADP and NAD activities.*

1) were pooled separately from those fractions showing high specificity towards NAD (peak II). The $NADH_2$ oxidase which usually interfered with crude enzyme assays was eluted in the fractions preceding the NADP-specific enzyme (with the yellow flavoprotein band). When enzymes free of AMP were required, all operations were conducted in Tris chloride-glutathione buffer (TG).

Stage 3. Ammonium sulfate fractionation. The NADP-specific GDH was concentrated by precipitation with solid $(NH_4)_2SO_4$ (65% saturation) at 5 C for 12 hr. The NAD-specific GDH was enriched, and some of the contaminating NADP-specific GDH was removed by differential precipitation with solid $(NH_4)_2SO_4$ (45% saturation) for 12 hr. The precipitates were recovered by centrifugation, and dissolved in small volumes of 0.1 M TGA buffer, *p*H 7.5. The heat step of Stage 1 was repeated on both enzymes, and the residue was removed by centrifugation at 48,000 × *g* for 10 min. The supernatant fluid was dialyzed for 6 hr against 4 liters of 0.05 M TGA buffer, with one change of the dialyzing solution.

Stage 4. Rechromatography. The partially purified GDH enzymes were rechromatographed separately by use of DEAE cellulose columns (1.0 × 15 cm), as described in Stage 2, and only 3-ml fractions were collected. The contamination of the NAD-GDH by residual NADP-GDH was completely removed as a separate peak, and *vice versa* (Fig. 1, inset). Those tubes showing either NADP-GDH or NAD-GDH activity as contaminants were discarded.

Stage 5. Rechromatography with $(NH_4)_2SO_4$. Both enzymes were individually precipitated with solid $(NH_4)_2SO_4$ (65% saturation) in the manner described in Stage 3. The enzymes were collected by centrifugation, dissolved in a small volume of 0.1 M TGA buffer, *p*H 7.5, and stored with a few drops of saturated ammonium sulfate solution at −20 C. Suitable portions were dialyzed overnight against 0.05 M TGA buffer, *p*H 7.5, at 5 C before use in kinetic analyses. A summary of the purification procedure is shown in Table 1.

Characterization of the GDH enzymes. The kinetic properties of the two enzymes differed considerably. The kinetics of the NAD-specific enzyme was influenced by AMP and ATP whereas the NADP-specific enzyme was unaffected by these substances (6). Data providing various kinetic constants for both enzymes have been reported elsewhere (H. B. LéJohn, I. Suzuki, and J. A. Wright, J. Biol. Chem., *in press*).

In the absence of AMP, saturation kinetics for glutamate and NAD were sigmoidal (Fig. 2), whereas the kinetics for α-ketoglutarate and $NADH_2$ were hyperbolic (Fig. 3). As illustrated in Fig. 2, the sigmoid functions could be modified to hyperbolic functions either by increasing the substrate concentrations or by the addition of AMP at 10^{-3} M. AMP inhibited the oxidation of $NADH_2$ at low substrate concentrations but was ineffective at high substrate concentrations (Fig.

TABLE 1. *Summary of purification of glutamate dehydrogenases from Thiobacillus novellus*

Fractionation step	Total vol (ml)	Total protein (mg)	Total units		Specific activity[b]		Recovery (%)[c]	
			NADP	NAD	NADP	NAD	NADP	NAD
1. Crude extract	100	6,020	192,140	—[a]	32	—	—	—
2. 1st heat treatment	85	3,000	180,000	102,900	60	34.3	100	100
3. 1st DEAE-cellulose								
(Peak I)	135	165	87,530	9,400	500.3	56.8	46	—
(Peak II)	125	168	13,180	80,000	78.6	357.6	—	77.6
4. 1st ammonium sulfate								
(0.60–0.65 saturation)	15	27.5	65,450	5,541	2,372	203.3	36.1	—
(0.40–0.45 saturation)	12	25.5	5,775	48,195	210	1,984	—	46.8
5. 2nd heat treatment								
(Peak I)	12.5	5.9	49,164	1,180	8,333	200	24.5	—
(Peak II)	9.5	5.25	6,300	36,908	1,200	7,030	—	35.8
6. 2nd DEAE-cellulose								
(Peak I)	25	4.75	36,200	—	7,564	—	20	—
(Peak II)	18	3.95	—	24,500	—	6,210	—	23.7

[a] Indicates that the specific activity could not be estimated due to interference from $NADH_2$-oxidase activity.

[b] Specific activity defined as enzyme units/mg of protein.

[c] Per cent recovery estimated from Stage 2, as the NAD-linked GDH activity is difficult to evaluate from Stage 1.

3b). For both NAD reduction and $NADH_2$ oxidation, then, AMP acted as an effector only at low substrate concentrations. The mechanism of enzyme activity and the sigmoid coefficient values were determined from detailed kinetic analysis (H. B. LéJohn, I. Suzuki, and J. A. Wright, J. Biol. Chem., *in press*).

Effect of pH on the kinetics. The kinetic assays were carried out at different *p*H values. When NAD-specific GDH was assayed at *p*H 8.0, rather

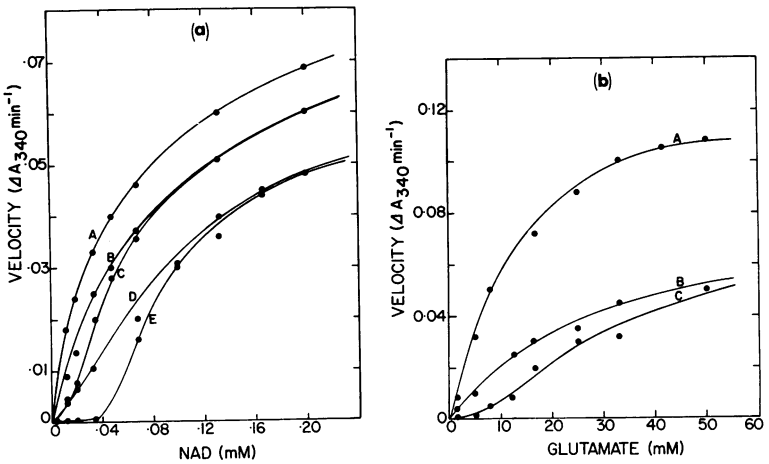

FIG. 2. *Effect of AMP and high substrate concentration on the kinetics of the NAD-specific GDH during the oxidative deamination of glutamate. (a) Velocity against substrate (NAD) plots with glutamate held at several fixed concentrations. The reaction mixture contained: (A) 66.66 mM glutamate with or without AMP; (B) 33.33 mM glutamate plus AMP; (C) 33.33 mM glutamate minus AMP; (D) 16.66 mM glutamate plus AMP; and (E) 16.66 mM glutamate minus AMP. (b) Velocity against substrate (glutamate) plots with NAD held at two fixed concentrations. The reaction mixture contained: (A) 1.33 mM NAD plus or minus AMP; (B) 0.067 mM NAD plus AMP; (C) 0.067 mM NAD minus AMP. The reactions of (a) and (b) were carried out in 0.2 M Tris chloride buffer, pH 9.5, with 50 μg of enzyme protein taken from Stage 4 (see Table 1) of the isolation procedure. Where indicated, AMP was added at 10^{-3} M.*

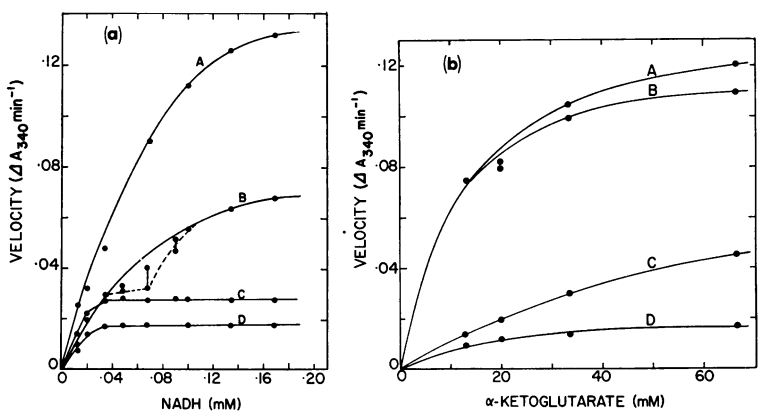

FIG. 3. *Effect of substrate concentration and AMP on the kinetics of the NAD-specific GDH during the reductive amination of α-ketoglutarate. (a) Velocity against substrate ($NADH_2$) plots with ammonia and α-ketoglutarate held constant at high (at least 5 × K_m) and low (less than K_m) concentrations. The reaction mixture contained: (A) 40 mM ammonia and 33.33 mM α-ketoglutarate, (B) 40 mM ammonia and 1.33 mM α-ketoglutarate; (C) 2 mM ammonia and 33.33 mM α-ketoglutarate; and (D) 2 mM ammonia and 1.33 mM α-ketoglutarate. (b) Plots of velocity against α-ketoglutarate concentration. $NADH_2$ was held at a constant high (5 × K_m) concentration and ammonia varied. Where indicated, AMP was present at 10^{-3} M concentration. The reaction mixture contained: (A) 0.33 mM $NADH_2$ and 80 mM ammonia; (B) 0.33 mM $NADH_2$, 80 mM ammonia, and AMP; (C) 0.33 mM $NADH_2$ and 1 mM ammonia; and (D) 0.33 mM $NADH_2$, 1 mM ammonia, and AMP. The assays of (a) and (b) were conducted in 0.2 M Tris chloride, pH 8.0, and each assay contained 20 μg of enzyme protein (Stage 4).*

than at *p*H 9.5, saturation kinetics for glutamate and NAD were hyperbolic rather than sigmoid. However, AMP still increased the apparent affinity of the enzyme for these ligands. The kinetics for $NADH_2$ and α-ketoglutarate were the same at *p*H 8.0 and at *p*H 9.5. The findings are shown in Fig. 4–5. Addition of AMP had no effect on the velocity of the reaction during $NADH_2$ oxidation (Fig. 5), but it increased the affinity of the enzyme for NAD (Fig. 4b). Increases in NAD or glutamate failed to produce the effects shown by AMP (unlike the results of Fig. 2).

The effect of ATP could be clearly demonstrated at *p*H 8. The results of an experiment in which ATP inhibited the oxidation of glutamate are shown in Fig. 4b. Apparently, the kinetic parameters became modified to sigmoid functions, a feature that was not evident even in the absence of AMP (Fig. 4a). In the absence of AMP, ATP had no influence on the reaction. This suggests that ATP may be a competitive inhibitor of AMP, although the possibility of different but interacting sites could not be excluded.

Heat stability. In addition to the chromatographic and kinetic behavior of the GDH enzymes, other criteria, including thermal inactivation, were used in characterizing these enzymes as two distinct proteins. Both enzymes were fairly stable to heat. The NAD-GDH retained 93% of its activity when heated to 55 C for 5 min. At 65 C, only 0 to 5% of the activity remained. The activity could not be restored by the addition of GSH. This indicated that the inactivation was due to changes other than labilization of free thiol groups. On the other hand, GSH could restore some of the activity in enzymes that had been inactivated by isolation and storage in the cold in the absence of GSH.

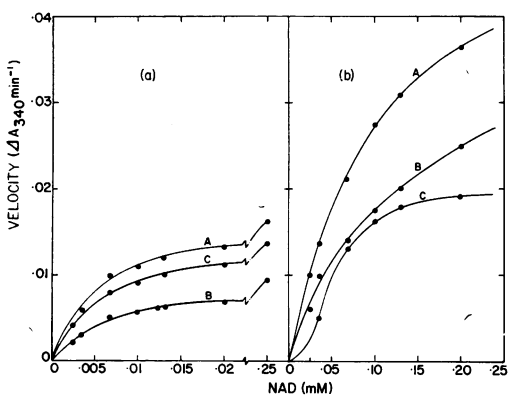

FIG. 4. *Effect of AMP and ATP on the reaction kinetics of the NAD-specific GDH at pH 8.0. (a) The reaction mixture consisted of 50 μg of enzyme protein in 0.2 M Tris chloride buffer, pH 8.0, and (A) 66.66 mM glutamate, (B) 33.33 mM glutamate, (C) 66.66 mM glutamate plus ATP. (b) The reaction mixture consisted of the same components as in (a) except that AMP was added to A, and B and C contained ATP in addition to AMP.*

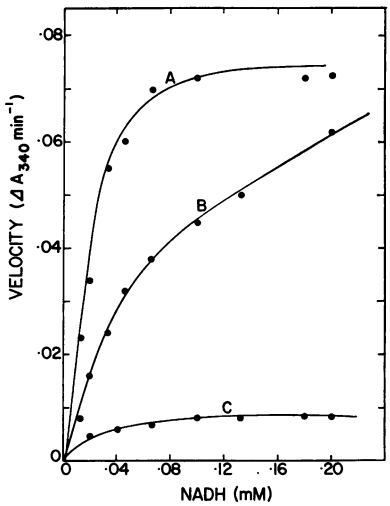

FIG. 5. *Effect of AMP on the kinetics of the NAD-specific GDH during the reductive amination of α-ketoglutarate at pH 9.5. The reaction mixture consisted of 50 μg of enzyme protein in 0.2 M Tris chloride buffer, pH 9.5, and (A) 40 mM ammonia and 33.33 mM α-ketoglutarate, with or without AMP; (B) 40 mM ammonia and 1.33 mM α-ketoglutarate, with or without AMP; and (C) 4 mM ammonia and 1.33 mM α-ketoglutarate, with or without AMP.*

The NADP-GDH remained stable to 65 C when only 7% of the activity was lost. At 77 C, it was completely inactivated. The thermal inactivation curves for both enzymes are shown in Fig. 6.

pH optima. Under the conditions of assay used, maximal activity for the oxidation of $NADPH_2$ was observed at *p*H 7.5, whereas for $NADH_2$ maximal activity occurred at *p*H 8.0. Reduction of NADP was maximal between *p*H 9.0 and 9.5, and reduction of NAD between 9.5 and 10.0 (Fig. 7).

Substrate specificity. Of the many amino acids and keto acids (L-aspartate, L-alanine, L-valine, L-leucine, oxalacetate, pyruvate, α-ketobutyrate, α-ketoisocaproate, and α-ketoisovalerate) tested, the enzymes reacted only with L-glutamate and α-ketoglutarate. No transhydrogenase activity was demonstrated by the purified enzymes.

Coenzyme specificity. The NADP-GDH showed no activity when NAD or $NADH_2$ was substituted for NADP and $NADPH_2$. The same strict specificity was demonstrated by the NAD-GDH enzyme.

Induction and repression of GDH enzymes. Since two separate enzymes catalyze the same reac-

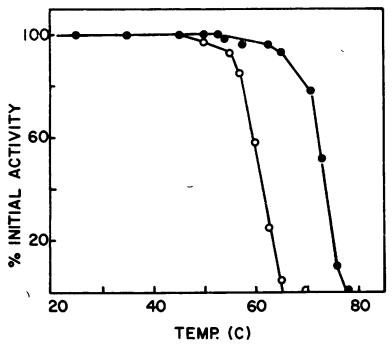

FIG. 6. *Thermal inactivation curves of NAD-GDH (○) and NADP-GDH (●) isolated from Thiobacillus novellus. The purified enzyme fractions were heated to the temperatures indicated, maintained at that temperature for 3 min, then rapidly chilled to 5 C in an ice-salt bath before assaying for activity. The same enzyme fraction was used in all assays and heat treatment. All assays were conducted with 10 μg of enzyme protein in 0.2 M Tris chloride, pH 7.5 (●) and pH 8.0 (○).*

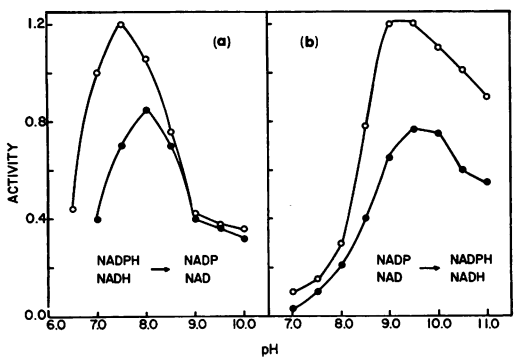

FIG. 7. *Effect of pH on the GDH activities. (a) The assay of NADPH (○) and $NADH_2$ (●) oxidation; (b) assay of NADP (○) and NAD (●) reduction. The reaction system in (b) contained five times more enzyme (50 μg) than (a). In each case, the pH of the reaction mixture (Materials and Methods) was determined prior to the addition of enzyme and at the end of the assay, which was carried out in 0.2 M Tris chloride buffer.*

tion, viz., the reversible breakdown of glutamate into α-ketoglutarate in *T. novellus*, we wanted to determine whether one or both enzymes are regulated by the physiological environment of the cell. For these studies, enzyme assays were conducted under conditions specified in Materials and Methods. The assay medium was designed to give optimal activities of both enzymes.

Autotrophic cells were examined during early log phase of growth for their GDH activities. The NADP-specific enzyme was found to be five times more active than the NAD-specific enzyme. By late log phase, the relative activities of the NADP- and NAD-linked enzymes had changed to a ratio of 2:1. The results are shown in Table 2. When the autotrophic cells were grown under heterotrophic conditions (7), the level of activities of the two enzymes differed widely depending on the carbon source used. Glutamate-grown cells had the highest level of the NAD-specific GDH. This level was higher than that of the NADP-specific GDH which was partially repressed. Histidine-, aspartate-, and arginine-grown cells showed significantly low levels of the NADP-specific enzyme. Compared to the autotrophic cells, less than 2% of the level of activity of the NADP-specific enzyme was found in these three carbon sources. This is understandable in the case of histidine and arginine, both of which must be broken down via glutamate during oxidation. This explanation does not apply to aspartate-grown cells. If it is argued that the glutamate requirement of the cell is satisfied by its synthesis from aspartate and α-ketoglutarate through the glutamate-aspartate transamination reaction, then some change in the activity of glutamate-aspartate transaminase may be expected. This was not found. All the heterotrophs had strong glutamate-aspartate transaminase activity.

The levels of the NADP-specific enzyme were similar in cells grown in glucose, alanine, and succinate. However, glycerol-, citrate-, and lactate-

TABLE 2. *Specific activities of NAD- and NADP-specific glutamate dehydrogenases of Thiobacillus novellus grown on various organic substrates*

Substrate	Concn (M)	Specific activity[a] of glutamate dehydrogenase		Ratio[b] NAD:NADP	Growth constant (μ)[c]
		NAD-linked	NADP-linked		
No addition					
Early log phase	—	5.0	25.0	0.2:1	20
Late log phase	—	22.0	49.0	0.45:1	
L-Alanine	0.03	20.7	45.0	0.47:1	8.5
L-Arginine	0.03	16.0	1.0	16.0:1	7.5
L-Aspartate	0.03	16.7	1.0	16.7:1	10
L-Histidine	0.03	18.2	1.0	18.2:1	10
Glutamate	0.05	30.8	17.5	1.74:1	8
Glucose	0.05	24.0	52.0	0.46:1	5
Glycerol	0.05	22.0	26.0	0.84:1	5
Citrate	0.025	24.6	24.6	1.0:1	9
Lactate	0.05	4.8	23.0	0.28:1	5.5
Succinate	0.025	6.7	61.3	0.11:1	10

[a] Specific activity estimated as enzyme units per mg of protein.

[b] All ratios normalized with respect to the specific activity of the NADP-specific GDH.

[c] μ = Exponential growth rate, hr^{-1}.

grown cells had lower levels of the NADP-specific GDH. The NAD-specific GDH was found at a fairly constant level in all cases, with the exception of lactate- and succinate-grown cells. No obvious correlation could be found between growth constants and enzyme levels.

DISCUSSION

In the chemoautotrophic bacteria, glutamate probably serves as a donor of amino groups and must be incorporated, either wholly or in part, into protein and nucleotides. Glutamate should then be functionally important as a cross-link between carbohydrate, amino acid, and nucleic acid metabolism in these organisms. When *T. novellus* is grown autotrophically, fully two-thirds of the glutamate dehydrogenase in the cell is of the NADP-variety. The activity of NAD-GDH increases with age, and is presumably regulated by the intracellular concentration of glutamate. During the conversion from autotrophic to heterotrophic cells by use of glutamate or some suitable carbon substrate, relatively high levels of the NAD-variety of glutamate dehydrogenase may appear. The level of this induced enzyme varies according to the carbon source. From the data presented, one can tentatively conclude that amino acids (histidine and arginine), which are metabolized and degraded via glutamate into the Krebs acid cycle, induce higher levels of the NAD-variety of GDH. During growth on glucose, succinate, or alanine, the cells form more $NADPH_2$-specific varieties of GDH than when grown on citrate or lactate. However, other compounds such as citrate and lactate, reduce the activity of the NADP-GDH.

A tentative conclusion that can be drawn from the effect of ATP is that this substance antagonizes the stimulatory action of AMP either by competing for the same binding site or by altering the enzyme form to that present at *p*H 9.5, or by both. It is conceivable that the enzyme may be present in at least three states, one of which shows optimal activity. At *p*H 9.5, both AMP and a high concentration of substrate can induce the active form. At *p*H 8.0, only AMP can induce this change. ATP, however, is able to reduce the activating effects of AMP and return the enzyme to the state that is present at *p*H 9.5. This explanation is difficult to apply to the results of the reverse reaction, unless one assumes that NAD and NADH are sufficiently different molecules and may not necessarily induce the same response.

Recent knowledge of regulation and energy economy suggests that when different enzymes that catalyze the same reaction are found within the same organism, such enzymes must possess different functions. A widely held view is that the NAD-specific dehydrogenases generate $NADH_2$ which is then oxidized via the electron-transport chain to produce ATP (4). $NADPH_2$, produced from the NADP-specific dehydrogenases, is used principally as the reducing power for biosynthesis. Some support for this concept has been obtained from the work of Kaplan and his co-workers (5) on the isocitric dehydrogenases of animal and microbial origin and from the kinetic analyses of the same enzymes by Atkinson et al. (1). We have obtained kinetic data which indicate that the allosteric NAD-GDH in *T. novellus* is regulated by the presence of ADP, AMP, and ATP (6). In the absence of AMP or ADP, the equilibrium of the reaction of the NAD-specific GDH may shift in favor of glutamate synthesis from α-ketoglutarate as shown schematically below:

$$\text{Glutamate} + \text{NAD} \underset{}{\overset{\text{(AMP or ADP)}}{\rightleftharpoons}} \alpha\text{-ketoglutarate} + \text{NADH}_2\,(\text{ATP or No effector}) + \text{NH}_4^+$$

ADP and AMP act as positive effectors and modulate the catalytic behavior of the enzyme so that glutamate breakdown is increased.

ACKNOWLEDGMENT

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